Protocol for expressing circularized TALEs tagged with GFP

PCR

Material:

10 µl 5 x HF Phusion Buffer (with $MgCl_2$)
5 µl dNTPs
2.5 µl forward Primer
2.5 µl reverse Primer
1 µl Template (TCR5 H2) – the NTH3 HA-GFP is going to be amplified
0.5 µl Phusion Polymerase 2 U/µl
28.5 µl H_2O
Thermocycler

Method:

Two different PCR programs were used.

Program 1:

98 °C – 2 min
98 °C – 10 sec
60 °C – 10 sec
72 ° C – 30 sec
72 °C – 5 min
35 x

Program 2:

98 °C – 2 min
98 °C – 10 sec
66 °C – 10 sec
72 ° C – 30 sec
72 °C – 5 min
35 x

Agarose gel

Material:

50 ml TAE buffer
500 mg Agarose
6 µl RedSave
7 µl 1 kb DNA ladder
20 µl Sample with 5 µl 5x loading buffer (20 µl loaded on agarose gel)

Method:

1 % agarose gel load with Samples ran for 20 minutes at 120 V.

Team TALebots

Gel elution

Material:

Gel Elution Kit
Agarose gel
Scalpel
Heating block

Method:

Cut the fragment out of the gel. Add 300 µl QG buffer and incubate for 10 min at 50 °C. Add 100 µl Isopropanol and transfer everything to the column. Centrifuge 1 min at max. rpm, discard the supernatant and add 400 µl QG buffer. Centrifuge again 1 min at max. rpm. Add 700 µl PE buffer, centrifuge 1 min at 1500 rpm, discard the supernatant and centrifuge again to dry the column for 1 min at max rpm.
Replace the column in a new 1.5 ml reaction tube and add 15 µl EB buffer or H2O tot he column. Incubate for one minute and centrifuge for 1 min at max rpm. Store the Sample at – 20 °C.

Assembly: Golden Gate Cloning

Material:

5 µl DNA (from gel elution - GFP NTH3 –HA)
1 µl SmaI
1 µl T4 Ligase
1 µl Vector (Puc57)
1 µl ATP
1 µl tango buffer

Method:

Incubate the solution for one hour at room temperature.

Heat shock transformation

Material:

15 µl of Cut Ligation reaction
50 µl competent E.coli cells (Top10)
200 µl LB Medium
Antibiotic
Waterbath
Incubator (37 °C)

Method:

Add 15 µ of the assembly reaction to 50 µl competent cells. Incubate for 20 minutes on Ice. Heat shock at 42 °C for 45 seconds. Back on ice add 200 µl LB Medium without antibiotic and incubate for 30 minutes up to 1 hour at 37 °C.
Plate the transformation on LB with antibiotic (in this case chloramphenicol) and incubate overnight at 37 °C.

Transformation with electroporation

Material:

50 µl competent E.coli cells (BL21DE3)
15 µl plasmid DNA
Electroporator
LB plates with chloramphenicol
200 µl LB

Method:

Add 50 µl competent cells and 15 µl plasmid DNA in a cuvette and use the electroporator for an electroshock. Add 200 µl LB in the cuvette and mix it. Everything in a 1.5 ml reaction tube and incubate for 30 minutes up to 1 hour at 37 °C. Plate on LB with chloramphenicol and store at 37 °C overnight.

Overnight cultures

Material:

5 ml LB medium
5 µl antibiotic (Chloramphenicol)
1 colony from the plates from the transformation with the assembly product.

Method:

Add 5 µl antibiotic and one colony to 5 ml of LB medium and incubate at 37 °C overnight.

Plasmid isolation

Material:

QIA Prep Spin Miniprep Kit
4 ml overnight culture

Method:

Centrifuge 4 ml of overnight culture for 3 minutes at 13000 rpm. Discard supernatant and add 250 µl P1. Resuspend the pellet and add 250 µl of P2. Invert for 4 to 6 times. Add 350 µl of N3 and invert again 4 – 6 times. Centrifuge for 10 minutes at 13000 rpm.
Now move the supernatant on the QIA Prep Spin column. Centrifuge for 1 minute. Remove the flow through and add 750 µl of PE buffer. Centrifuge one minute at 13000 rpm, remove the flowthrough and centrifuge again for 1 minute at 13000 rpm.
Put the column in a new 1.5 ml reaction tube and add 50 µl EB. Incubate 1 minute and centrifuge 1 minute. Remove the column and keep the flow through at -20 °C.

Control digest

Material:

2 µl 10x reaction buffer
0.5 µl XbaI
0.5 µl PstI
500 ng DNA
Add 20 µl H_2O

Method

Incubate the reaction for 1 hour at 37 °C and stop the reaction for 10 minutes at 65 °C.

Preparation of electrocompetent cells

Material:

Overnight culture
Ice
Centrifuge
10 % Glycerin
H_2O

Method:

Grow the cells until they reach an optical density at 600 nm of 0.5 – 0.6. Incubate the bacteria 20 min on ice. Centrifuge 10 min at 4 °C and discard the supernatant. Add 20 ml cold glycerin (10 %) and resuspend the pellet. Centrifuge again 10 min at 4 °C. Add 20 ml cold H_2O and centrifuge for 10 min at 4 °C. Discard the supernatant and add 10 ml cold glycerin (10 %). Resuspend the pellet and centrifuge at 4 °C for 10 min. discard the supernatant and resuspend the pellet in 2 ml 10 % cold glycerin. Take 100 µl aliquots into 1.5 µl reaction tubes. Froze them in liquid nitrogen and store at - 80 °C or use them directly for transformation.

Expression

Material:

2.5 ml from each overnightculture (BL21 + Ax7L-DS, BL21 + Ax7R-RR, BL21 + Ax7L-scFOK, BL21 + Ax7R-scFOK)
100 ml LB (for each culture)
Antibiotic
IPTG (2 mM)
Centrifuge

Method:

Add 2.5 ml from each overnight culture to 100 ml LB. Measure the optical density at 600 nm. Let the bacteria grow until they reach an optical density about 0.5. Now add 2 mM IPTG to induce the expression of the TALE protein.
After 1 hour and 30 min, stop expression and centrifuge for 10 minutes. Store at -80 °C.

Strep Tag Purification

Material:

Pellet from Protein expression
TE buffer
PMSF
centrifuge
ultrasonic
Twin Strep Tag Purification Kit from Iba (0.2 ml column)

Method:

Take a little bit from expression pellet and resuspend in 2 ml TE buffer and 20 µl PMSF. Use the ultrasonic program: 30 sec ultrasonic and 30 sec pause 8 times.
Centrifuge the Samples 15 min at 4 °C and 20000 rpm
Now use the protocol from the Twin Strep Tag Purification Kit. First add 2 times of the column bed volume (CV, 0.2 ml) of Buffer W. Apply 0.1 up to 10 of the CV from the supernatant (from the centrifuged Samples) to the column.
When the liquid completely entered the column, add 1 CV washing buffer w. Repeat this step 4 times.
After finishing the washing step, add 0.5 CV washing buffer W. Repeat it 5 times until there are 6 elution Samples.
Regenerate the column three times with 5 CV regeneration buffer R.
Finish the regeneration by adding 2 times 4 CV washing buffer w and store the column at 4 °C with 2 ml washing buffer w.

TCA precipitation

Material:

Samples (from strep tag purification)
NaDOC
Ice
TCA
80 % Acetone
Centrifuge
Heating block

Method:

Mix 30 µl Samples with 3 µl NaDOC. Incubate for 10 min on ice. Add 3 µl TCA and incubate for 60 min on ice. Centrifuge for 20 minutes at 6500 rpm and 4 °C. Resuspend the pellet in 1 ml 80 % Acetone. Centrifuge at 6500 rpm and 4 °C for 10 min. Discard the supernatant and dry the pellet at 37 °C in a heating block.

SDS-PAGE

Material:

For 10 Gels:

separating gel (10 %):
- 27.25 ml H2O
- 33.25 Acrylamid (30 %)
- 25 ml Tris (1.5 M; pH 8.8)
- 1 ml APS (10 %)
- 1 ml SDS (10 %)
- 40 µl TEMED

stacking gel:
- 34 ml H2O
- 8.5 ml Acrylamid (30 %)
- 6.25 ml Tris (0.5 M; pH6.8)
- 0.5 ml APS (10 %)
- 0.5 ml SDS (10 %)
- 50 µl TEMED

TANK buffer:
- 30 g Tris
- 144 g Glycine
- 100 ml 10 % SDS

Samples:
- 10 µl with column purified Sample (or a little bit of the expression pellet)
- 10 µl Lämmli (protein loading buffer)
- 7 – 10 µl Page Ruler Prest protein ladder

Method:

For preparing an SDS gel, start with the separating gel. Bring everything together in this order: 1. H_2O, 2. Tris, 3. SDS, 4. Acrylamide, 5. APS, 6. TEMED; APS and TEMED every time at last! After adding them be fast. Pour it into the gel apparatus and cover it with 1 ml Isopropanol. Wait until it got solid. Then prepare the stacking gel. Get everything in the same order together (1. H_2O, 2. Tris, 3. SDS, 4. Acrylamide, 5. APS, 6. TEMED). Remove the Isopropanol and pour the stacking gel above the separating gel.

Prepare the Samples. Take 20 µl of each sample and add 20 µl loading buffer. Incubate for 10 min at 100 °C. Load 20 µl of each prepared Sample into the gel and run the gel at 150 V for 40 min or until the first band of the protein ladder reached the end of the gel.

Semi dry Western blot

Material:

Blotting chamber
Power supply
PVDF membran
6 filter paper
SDS Gel

Toubin:
5.81 g Tris
400 µl 10 % SDS
2.89 g Glycine
200 ml Methanol

Ponceau solution:
0.2 % Ponceau
3 % TCA
Store at room temperature

Blocking solution:
1 x RotiBlock
1 x PBST

Method:

Put the filter paper into the buffer, stack 3 filter papers, put the buffer soaked membrane on the filter paper, lay the gel on the membrane and stack again 3 filter papers soaked with buffer at the gel. Remove the air in the stack and run the Transfer at 5.5 V per 1 cm^2 for 40 min.
Incubate the membrane for 10 min in ponceau solution. Destain the membrane with H_2O and incubate the membrane at least for one hour in blocking solution.

Coomassie staining

Material:

Coomassie staining solution:
0.05 % Coomassie R250
25 % Isopropanol
10 % glacial acetic acid

Destaining solution:
10 % glacial acetic acid or H_2O

Method:

Add Coomassie to the gel at least for 20 min. Remove the coomassie staining solution and add the destaining solution or water until the redundant coomassie is gone.

Immunostain

Material: Pincette
Bowl for Immunostain
Rocker

10 x PBS:	2.56 g NaH_2PO_4 x H_2O
	14.90 g Na_2HPO_4
	87.66 g NaCl
	Ad 1 l H_2O pH 7.4
PBST:	PBS pH 7.4
	0.05 % Tween20
Blotto:	1 x RotiBlock
	1 x PBST
Substrate solution per membran:	6 ml 100 mM Tris pH 8.5
	30 µl Luminol (22.2 mg/ 500 µl DMSO)
	15 µl p-Cunaracid (7.4 mg/ 500 µl DMSO)
	10 µl 30 % H_2O_2

Antibody 1: StrepMAB – Classic cell culture supernatant IgG1 Anti – Strep – tagII monoclonal 2-1508-025 Iba Life Sciences

Antibody 2: ECL – Anti-mouse IgG Horseradish Peroxidase-linked whole antibody GE healthcare UK NA931V lot 9682503

Method:

Wash the membrane with PBST. Incubate the membrane with the first antibody (1:2000 in blotto) for one hour while shaking.
Wash the membrane 1 x short with PBST, 1 x 5 min with PBST.
Add the second antibody (1:10000 in blotto) and incubate for one hour while shaking.
Wash the membrane 1 x short with PBST, 1 x 5 min with PBST.
Incubate with substrate buffer for a short time (30 sec up to 20 min).

Team TALe bots

iGEM Chip

Material:

Spotter
Chip
Oligonucleotide
0.2 % SDS
H_2O
$NaBH_4$
Blocking solution

Method:

Convict the solutions on in the Lafontain plates (100 mM Oligonucleotides (10 µl) and 10 µl 2x Array it Micro Spotting) and spot the oligonucleotides on the plates.

After spotting incubate 3 min at UV-Crosslink. Incubate for 24 h at room temperature in a dark room.

For reducing: Incubate 2 min with 0.2 % SDS. Wash 2 times 1 min with H_2O. Incubate again 5 min with $NaBH_4$ and wash with cold H_2O 1 min. Incubate 1 min with 0.2 % SDS and wash 2 times with H_2O for one minute.

Dry the plate and block it with blocking solution at 42 °C for 45 min. Wash 5 min with H_2O and dry the plate again.